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Thyroid Hormone and Male Gonadal Function*

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I. Introduction

WHILE the effects of hyper- and hypothyroidism[1] in female gonadal functions are well established (1, 2), controversy exists regarding the impact of thyroid diseases on male reproduction. This is due to various reasons: 1) the apparent clinical irrelevance of signs and symptoms related to male gonadal function, compared with the systemic effects of hyper- and hypothyroidism; 2) the paucity of well controlled clinical studies, due to the fact that thyroid diseases are more common in females than in males; and 3) the demonstration in the 1950s that the adult male gonad of experimental animals is metabolically unresponsive to thyroid hormone (3). Since then, the concept that the testis is unaffected by iodothyronines became widely accepted. For this reason, the potential of thyroid hormone in the modulation of male reproductive functions was not determined for several years. Indeed, in experimental studies on the effect of thyroid hormone in various tissues, the testis has been used as a negative control. This review, in the light of clinical reports and experimental results, will refute this assumption. The body of the review consists of two parts. In the first part the effects of hyper- and hypothyroidism in man on gonadotropin release, sex steroid hormone metabolism, and testicular and sexual functions, both in adulthood and childhood, are discussed. In the second part, animal studies are reviewed. In the rat, which is the most used experimental model, the *in vivo* and *in vitro* effects of thyroid hormone on reproductive physiology are discussed. The last section summarizes studies on other animals, from primates to amphibians, and considers the role of the thyroid gland in regulating the seasonal pattern of reproduction.

II. Clinical Studies

A. Hyper- and hypothyroidism in adulthood

1. Gonadotropin release. In adult onset hyperthyroidism, basal levels of gonadotropins were normal, with LH and FSH responses to exogenous GnRH significantly greater in the untreated than in euthyroidism post treatment (4). To explain this finding, the authors hypothesized a direct effect of thyroid hormone on gonadotroph sensitivity to GnRH. The same mechanism has been suggested in hyperthyroid women and in women receiving T_3 or T_4 who showed increased LH and FSH responses to exogenous GnRH (5).

In myxedematous men a hypergonadotropic state was reported (6–8), although hypogonadotropic hypogonadism (7) or normal LH and FSH serum levels (9) were also found. The hypergonadotropic patients also showed an elevated biological/immunological LH ratio. The effect of thyroid hormone deficiency on gonadotropin secretion and bioactivity has been referred to as testicular resistance to gonadotropins (7); this is also supported by the reduced testosterone response to human CG (hCG), corrected by substitution therapy (8), in hypothyroid men.

The data herein summarized appear to be inconclusive,

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[1] The terms hyper- and hypothyroidism are used to denote significantly higher or lower, respectively, thyroid hormone levels respect to healthy subjects, due to various causes. Thyrotoxicosis and myxedema have been used only when specified by the quoted authors or to indicate severe hyper- or hypothyroidism, respectively. The term thyroid hormone is used to denote the two metabolically active iodothyronines, L-tetraiodothyronine or T_4 and L-triiodothyronine (T_3).

largely due to a lack of carefully controlled studies performed in an adequate number of patients. Moreover, the pituitary response to exogenous GnRH has not been standardized, and dramatic differences in individual responses are a common experience (10). In hyperthyroidism, the effect of altered steroid hormone *milieu* on the hypothalamic-pituitary axis should be evaluated (see below), together with the secretion of tropin α-chains, or the LH and FSH response to longer exposure to pulsatile administration of GnRH.

2. *Sex steroid hormone metabolism.* The stimulatory effect of T_3 on the hepatic production of sex hormone binding globulin (SHBG) (11, 12) is so specific that it can be considered one of the most effective biochemical markers of thyroid hormone peripheral action (13). Serum concentration of SHBG is increased in male hyperthyroidism (14–17) leading to a rise in circulating level of total testosterone (15, 17–21) and to a decrease in testosterone MCR (16, 18, 20, 22). However, the plasma level of free testosterone is not significantly different from normal (16–20, 23). This is in agreement with the lack of clinical consequences of the markedly elevated levels of total testosterone found in thyrotoxicosis. Moreover, the levels of 5α-dihydrotestosterone, an active form of androgen, were also increased with respect to controls, but rarely outside the normal range (19, 20).

Gynecomastia is present in 40–83% of hyperthyroid patients (19, 24–26). It is notable that one of Von Basedow's original patients had this symptom (27). Gynecomastia could result from the increased estrogen/androgen ratio associated with hyperthyroidism. In fact, it has also been demonstrated that SHBG binds testosterone preferentially, with twice the affinity of estradiol (16, 20, 28–31). In hyperthyroid patients, free estradiol serum levels were increased out of proportion to the rise in the binding globulin (19, 21). In addition to the indirect effect of thyroid hormone on estrogen metabolism *via* SHBG, an increased peripheral conversion of androgen to estrogen has been also reported (30). Last, serum progesterone was higher in hyperthyroid than in euthyroid males (17, 32).

Hypothyroid males may show decreased (7, 8, 33) or normal (34) SHBG serum levels, low total serum testosterone concentrations (7, 9), and normal levels of estradiol (9).

3. *Testicular function.* Direct effects of thyroid hormone on the human adult testis have never been demonstrated. However, a few indirect consequences of altered circulating thyroid hormone levels have been described. A single report suggests that hyperthyroidism may lead to a decrease in testicular volume (21) while severe hypothyroidism has been associated with testicular atrophy, again noted only in an isolated report (7). In two of 10 necroscopies performed in subjects with adult-onset myxedema, testicular atrophy was characterized by fibrous thickening of the basement membranes, by a decreased number of interstitial cells, or by some decrease in spermatogenesis (35). Hydrocele can also be present, along with other serous effusions, as a complication of severe hypothyroidism (36, 37). It does not require aspiration, because the treatment of myxedema with T_4 causes resolution.

It has been shown that hyperthyroid men have lower than normal sperm number, with normal motility (23). However, mean sperm density in the hyperthyroid men was recently found not different from control values, whereas the percent of forward progressive motility was significantly lower than controls (21, 38). Hypothyroidism has also been suggested as a cause of male infertility (39, 40), but this finding is a matter of considerable disagreement. In adult myxedema, normal semen analysis (41) or slight seminal abnormalities such as a decrease in ejaculated volume, in sperm progressive forward motility, and in the cumulative percentage of mobile forms were reported. However, these abnormalities were not intense enough to induce male infertility (9). There are only uncontrolled reports of the use of androgen-thyroid combination therapy in infertile men (42–44). Thus, to define the role of thyroid hormone, if any, on sperm production and metabolism, further well controlled studies are needed.

4. *Sexual behavior.* Anecdotally, an increase in libido is described in male thyrotoxicosis, while hypothyroidism is associated with diminished libido and impotence (6–8, 23). Furthermore, it has been reported that myxedema can be an important cause, in up to 5% of patients, of penile erectile dysfunction (45). The cause of these sexual symptoms is unknown. Considering the neuropsychic reactions to thyroid hormone excess or deficiency, the alteration of sexual behavior seems likely to be a nonspecific disease-related complaint, which can disappear when euthyroid state is achieved.

In conclusion, although adult hyper- and hypothyroidism have some effects on gonadotropin secretion and bioactivity, sex steroid hormone metabolism, and testicular function, they do not usually have a major clinical impact on male reproduction. This conclusion is further substantiated by molecular biology studies. The lack of any direct effect of thyroid hormone on the adult testis is consistent with the finding that until now only the *c-erbA*$_{\alpha 2}$ sequence, which encodes a thyroid hormone receptor isoform that does not bind the hormone (see *Section III.B*), has been cloned from a human adult testis cDNA library (46).

B. Hyper- and hypothyroidism in childhood

In girls affected by thyrotoxicosis, delayed sexual maturation has been demonstrated (47). Surprisingly, no studies have correlated male childhood hyperthyroidism with gonadal function.

Retardation of sexual development is anecdotally regarded as a cardinal feature of juvenile hypothyroidism (47, 48). However, in this case clinical reports exclusively concern girls. On the contrary, several well controlled studies in boys have demonstrated that prepuberal thyroid failure can occur in association with precocious sexual development. In male hypothyroidism the clinical picture of precocious puberty is characterized by enlargement of the testes, without virilization (Fig. 1). The testicular size (longitudinal and horizontal axes) has been measured by a caliper and the testicular volume (milliliters) has been evaluated by a Prader orchidometer. These parameters have been staged by comparison to normal stages of development (49). Fifty-seven cases of prepuberal hypothyroidism have been reported in boys (Table 1), and more than 75% had testicular size measurement compatible with macroorchidism. Prepuberal males affected by

FIG. 1. Patient of 6.5 yr with acquired hypothyroidism. Note short stature for age, plumpness, and large testes without pubic hair. Biopsy of the testes revealed beginning spermatogenesis with spermatocytes and spermatids and a few Leydig cells. [Reproduced with permission from Z. Laron *et al.*: *Acta Paediat Scand* 59:317–322, 1970 (55). © Scandinavian University Press.]

primary hypothyroidism have been reported with normal (60, 61) or, more frequently, elevated immunoreactive FSH and LH (55–57, 62), and with total serum testosterone in the prepuberal range (57–60, 62, 64). In particular, in the largest studies, FSH serum levels were increased in all subjects, while LH was elevated only in 50% of patients (56, 62). This is a reversal of the pattern seen in normal children in early puberty, where LH predominates over FSH (65). An altered response of LH and FSH to GnRH (58, 60, 64, 66) and a tonic, as opposed to episodic, release of LH were also demonstrated in prepuberal male hypothyroidism (66).

The rare testicular biopsies performed both before and after puberty may clarify the nature of the gonadal damage associated with prepuberal hypothyroidism. Testis specimens obtained before puberty showed a predominance of tubular compartment, characterized by an early onset of spermatogenesis with few spermatocytes and spermatids, but no increase in the number of Leydig cells (53, 55). On the contrary, biopsies performed in adult patients affected by untreated, juvenile-onset hypothyroidism showed tubular walls with fibrosis and hyalinization, fibroblastic proliferation, and peritubular and interstitial fibrosis, with sparse Leydig cells (52, 64). Thus, instead of enlarged tubular compartment, as in the early phases of prepuberal onset hypothyroidism, the histological *scenario* of the disease encompassing puberty appears to be that of testicular atrophy and involution. With thyroid hormone replacement, the testes can decrease in size (53, 55–57) or remain macroorchid (55).

The pathogenesis of "precocious puberty" in hypothyroidism remains uncertain, even though different theories have been proposed: 1) it may result from early maturation of hypothalamic-pituitary-gonadal axis, but this does not seem to be the case, in view of the absence of virilization. 2) Van Wyk and Grumbach (67) suggested in a clinical study performed in girls that the syndrome resulted from increased secretion of gonadotropins as a consequence of an "overlap" in negative feed-back regulation with TSH. 3) The interaction of TSH with the human FSH receptor has been explored *in vitro*, indicating that TSH at high concentrations can bind to the FSH receptor and elicit a cAMP response (68). Thus, high levels of TSH, as in primary hypothyroidism, may overstimulate the seminiferous epithelium. 4) Barnes *et al.* (56) have suggested that hypersecretion of PRL, found in some hypothyroid boys with macroorchidism, is the primary pathogenetic factor leading to precocious sexual development. However, Castro-Magaña and co-workers (62), who studied nine boys with severe longstanding primary hypothyroidism associated with macroorchidism, concluded that testicular enlargement is the result of continuous FSH stimulation. In fact, no correlation was found between the degree of hyperprolactinemia and testicular size, while a linear correlation between testicular volume and FSH plasma levels at different pubertal stages has been demonstrated. 5) The mechanism of increase in FSH, but not LH, release has been related to what occurs in premature thelarche, in which it is suspected that a slight activation of GnRH pulse generator is able to increase FSH, but not LH, secretion (69). 6) A direct effect of thyroid hormone deficiency on the immature seminiferous epithelium cannot be ruled out. The abnormality in testicular development is suggested both by the selective activation of seminiferous epithelium and by the enhanced amplitude and frequency of FSH pulses (70). The specific tubular alteration found in juvenile myxedema can be related to a lack of the effects of thyroid hormone on testis maturation (as demonstrated in animals, *vide infra*).

The term "true precocious puberty" is not appropriate for boys with hypothyroidism and macroorchidism in view of the absence of the activation of the hypothalamus-pituitary-gonad axis (71). Furthermore, it cannot be considered a "pseudoprecocious" or "incomplete" puberty, since there is no autonomous androgenic hypersecretion. Thus, for the selective activation of seminiferous epithelium, the term "macroorchidism of hypothyroidism" is preferred.

Since modern histological analysis is not available in macroorchidism of prepuberal hypothyroidism, it is not clear whether germ cells proliferate and differentiate more than in the normal testis or whether the enlargement of the seminiferous tubule is due to an increase in Sertoli cell number, or both. However, in view of the animal data (see

TABLE 1. Case reports of male juvenile hypothyroidism in which pubertal development has been studied

Ref.	No. of patients	Diagnosis (n)	Macroorchidism (n)	Sexual hair (n)	Gonadotropin and testosterone (n)
50	1	Primary hypothyroidism (1)	Yes (1)	Yes (1)	nd
51	1	Cretinism (1)	Yes (1)	No (1)	nd
52	5	Thyroid insufficiency (5)	Yes (2) No (3)	nd	nd
53	7	Idiopathic hypothyroidism (7)	Yes (7)	No (7)	↓FSH (7) ↓LH (7)
54	6	Maldescent of thyroid gland (6)	nd	No (1)	nd
55	4	Congenital (2) and acquired hypothyroidism (2)	Yes (4)	No (2) Yes (2)	↑FSH (1) ↑LH (1)
56	9	Acquired idiopathic hypothyroidism (4) Hashimoto (4) T_4 biosynthesis defect (1)	Yes (9)	No (9)	↑FSH (9) ↑LH (4)
57	1	Acquired hypothyroidism (1)	Yes (1)	No (1)	↑↑FSH (1) ↑LH (1) —T (1)
58	1	Acquired hypothyroidism (1)	Yes (1)	No (1)	↑FSH (1) ↑↑LH (1) —T (1)
59	1	Sublingual gland (1)	Yes (1)	No (1)	↑FSH (1) ↑↑LH (1) —T (1)
60	2	Acquired hypothyroidism (1) Goiter (1)	No (2)	Yes (2)	—FSH (2) —LH (2) —T (2)
61	2	Primary hypothyroidism (2)	No (2)	Yes (2)	—FSH (2) —LH (2)
62	15	Autoimmune thyroiditis (15)	Yes (9) No (6)	No (9) Yes (6)	↑↑FSH (9) ↑LH (9) —FSH (6) —LH (6) —T (15)
63	1	Primary hypothyroidism (1)	Yes (1)	nd	↑FSH (1) —LH (1)
64	1	Chronic lymphocytic thyroiditis (1)	Yes (1)	No (1)	↑LH (1) —FSH (1) —T (1)
Total	**57**		**Yes 38** **No 13**	**No 34** **Yes 13**	**↑FSH 23** **↑LH 18** **—T (21)**

References have been chronologically listed. The diagnosis is that reported by the authors. ↑, Increased; ↓, decreased; —, not different from aged-paired controls; nd, not determined.

Section III.D), it can be inferred that low levels of thyroid hormones before puberty affect the timing of tubular differentiation and development. Longer lasting deficiency of thyroid hormone encompassing the pubertal crisis will lead to degeneration and fibrosis of tubule and interstitium. A more direct comparison between humans and experimental animals is speculative, since in human beings the proliferative and differentiative pattern of the seminiferous epithelium before puberty is not fully characterized. However, the finding that in men, as in rats, the numerical density of Sertoli cell declines with advancing postnatal age (72) suggests that also in men thyroid hormone deficiency may extend the period of Sertoli cell proliferation beyond the time when mitosis ceases.

In a review of the clinical literature, it appears clear that male reproductive function is not substantially affected when hyper- or hypothyroidism occurs after puberty. On the contrary, prepuberal hypothyroidism is associated with macroorchidism histologically characterized by enlargement of tubular compartment. The apparent low prevalence of this condition may be the result of poor awareness of its existence. The clinical relevance of the adverse effects of thyroid hormone deficiency on male reproductive functions is further emphasized in view of the very large number of prepuberal subjects at risk of hypothyroidism as a result of living in iodine-deficient environments. It should be considered that thyroid hormone deficiency in the early postnatal period, in addition to causing goiter, short stature, and various degrees of intellectual retardation (73), affects tubular development of male gonads. Furthermore, antithyroid therapies in prepuberal males (74) should be carefully evaluated for possible adverse effects on reproductive function due to overtreatment producing hypothyroidism.

III. Studies in the Rat

A. Thyroid hormone effects on male reproductive axis

The influence of thyroid status on anterior pituitary hormones has been extensively studied *in vivo* and *in vitro* in the

rat. Gonadotrophs exhibited an increase in their granular content upon thyroid hormone administration (75). FSH serum levels were reduced after chronic T_3 administration in adult (76–78) and in prepuberal rats (78, 79), while acute thyroid hormone treatment in the perinatal period induced a slight increase in FSH serum levels (80). Because no changes were documented in gonadotropin β-chain mRNA upon T_3 treatment (81), the site of action of this effect is to be considered posttranscriptional.

Thyroidectomy or goitrogen feeding reduces the number and size of gonadotropes (75), concomitantly with a decrease in serum levels of LH and FSH in prepuberal age (82–88). This effect was more pronounced on FSH than LH. Hypothyroidism induced in adult animals showed normal (89, 90) or lower (91, 92) FSH levels. Hypothyroidism did not affect the level of the FSH β-chain mRNA in the pituitary (81).

Total serum testosterone levels were found unchanged with respect to controls in rats made hypothyroid both in prepuberal stage (87, 93, 94) and after puberty (90, 94–96). Only two papers report a decrease in serum testosterone in rats thyroidectomized before (85) or after puberty (91). Leydig cells isolated from hypothyroid rats (97) or from adult rats that had been neonatally hypothyroid (98) produce less testosterone *in vitro* compared with controls. However, this effect is counterbalanced by the increase in the number of adult Leydig cells found in hypothyroid rats (98), producing no net change in peripheral total testosterone levels.

B. Thyroid hormone receptors (TRs)

The different biological effects induced by T_3 are not all derived from a common event; they can arise from the interaction of the hormone with multiple cellular sites. In fact, specific binding sites have been detected in plasma membrane, in cytosol, in mitochondria, in the nuclear envelope, and in the nucleus (Ref. 99 and references therein). Nuclear TRs are proteins that bind T_3 with high affinity [dissociation constant (K_d) = 10^{-9}-10^{-10} M] and specificity. TRs, which are currently thought to underlie most actions of T_3 and T_4, are tightly associated with chromatin and present in low abundance in almost all rat tissues (Ref. 100 and references therein). The thyroid hormone/TR complex triggers responsive genes by binding to specific sequences in their regulatory regions. The genes so affected influence metabolism, growth, development, metamorphosis, and differentiation (Ref. 101 and references therein). TRs are encoded by two different genes, α and β, which have been sequenced in human, rodent, avian, and amphibian species (102, 103). The primary transcript of each receptor gene is alternatively spliced, generating receptors α_1, its nonhormone binding variants α_2 and α_3, and the β_1-β_2 isoforms. The proteins translated *in vitro* from $TR_{\alpha 1}$, $TR_{\beta 1}$, and $TR_{\beta 2}$ mRNAs bind T_3 with affinity comparable to that of native TR. $TR_{\beta 2}$ is mainly present in pituitary, in some brain areas (104) and, at low levels, in other organs (105). The relative expression of the two major TR genes and the distribution of their products vary from tissue to tissue and during different stages of development (104–108). The relative importance of $TR_{\alpha 1}$ *vs.* $TR_{\beta 1}$ in mediating thyroid hormone nuclear effects is not clear. Many authors have tried to answer this question using *in vitro* expressed TRs and mammalian cells overexpressing cotransfected TRs and reporter gene constructs (reviewed in Ref. 109). However, determination of the relative physiological importance of $TR_{\alpha 1}$ and $TR_{\beta 1}$ requires *in vivo* corroboration. The physiological significance of $TR_{\alpha 2}$ and $TR_{\alpha 3}$ is unknown because they do not bind the hormone, but their structural similarities with TRs suggest that they might function as modulators of thyroid hormone action. The genomic *locus* of c-*erb*A_α, the protooncogene encoding for the TRα and their variants, transcribes another mRNA, but in part from the opposite DNA strand. Transcription of this mRNA, called Rev-*erb*A_α (Rev), continues downstream from the 3'-end of α-gene transcription unit, corresponding to the site of differential splicing of $TR_{\alpha 1}$/$TR_{\alpha 2}$. It has been suggested that the presence of a naturally occurring antisense RNA may modulate TR expression by the formation of heteroduplexes with $TR_{\alpha 2}$ (103).

Adult rat testis is devoid of T_3 nuclear binding activity (110, 111) and of the α_1, β_1, and β_2 mRNA isoforms, while it expresses the α_2 mRNA (106, 112–118). However, Palmero and fellow researchers (119) found that T_3 specifically binds with high affinity and low capacity to nuclei isolated from Sertoli cell cultures from the immature rat. After this report, we demonstrated in Sertoli cell nuclei that the concentration of nuclear thyroid hormone-binding sites changes during gonadal development, being maximally expressed in the fetus and in the early postnatal life, decreasing significantly throughout the prepuberal period, to be virtually absent in the adult (111). Germ and interstitial cells appear devoid of appreciable T_3-binding activity, confirming that the major target of thyroid hormone in testis is the somatic cell of the seminiferous epithelium.

Since in most tissues the level of TR_β appears to correlate better with the nuclear binding of thyroid hormone than does the amount of $TR_\alpha 1$ (106, 114), TR_β is thought to encode the "true" TR. For this reason, we measured the expression of TR forms and isoforms in testis at various stages of development (Table 2). We confirmed that not only adult, but also fetal, neonatal, and prepuberal testis is devoid of TR_β mRNA. $TR_{\alpha 1}$ mRNA is expressed from fetal through prepuberal stages while it is absent in adult testis (118, 120). The amount of $TR_{\alpha 1}$ mRNA correlates well with thyroid hormone nuclear binding sites previously demonstrated in the developing testis (Fig. 2). $TR_{\alpha 2}$ is constitutively expressed from fetal to adult life at higher levels than $TR_{\alpha 1}$ (118), while $TR_{\alpha 3}$ is absent. Finally, Rev mRNA appears to be developmentally regulated: it is absent in fetal testis, first appears in the prepuberal period, and is maximally expressed in adult male gonad

TABLE 2. Ontogenetic pattern of TR expression during rat testis development

	Fetal (19 dpc)	Perinatal (1–5 dpn)	Prepuberal (15–20 dpn)	Adult (60 dpn)
$TR_{\alpha 1}$	++	+++	+	−
$TR_{\alpha 2}$	++++	+++++	−+	+
$TR_{\alpha 3}$	−	−	−	−
Rev	−	−	++	−−−−
$TR_{\beta 1}$	−	−	−	−
$TR_{\beta 2}$	−	−	−	−

dpc, Days *post coitum;* dpn, days *post natum*. Data were compiled from Refs. 111, 118, and 121.

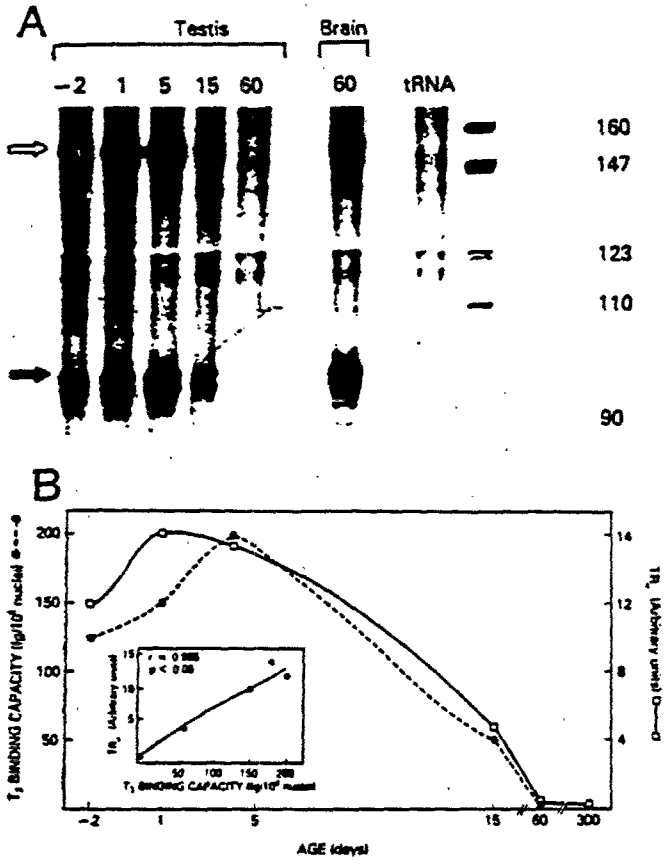

FIG. 2. Expression of $TR_{\alpha 1}$ and $TR_{\alpha 2}$ mRNAs and nuclear thyroid hormone binding capacity during rat testis development. A. Expression of $TR_{\alpha 1}$ and $TR_{\alpha 2}$ mRNAs. RNase protection assay was carried out using an antisense complementary RNA (riboprobe) derived from the C terminus of c-*erb*A_α sequence. Adult brain from a 60-day-old rat served as positive control, while yeast tRNA has been used as a negative control. Size markers are also shown. *Open arrow*, the 158-base fragment corresponding to $TR_{\alpha 1}$; *filled arrow*, the 98-base fragment corresponding to $TR_{\alpha 2}$. B. Comparison of the levels of $TR_{\alpha 1}$ mRNA (*solid line*) and T_3 nuclear binding capacity (*broken line*) (111) during rat testis development. The correlation between $TR_{\alpha 1}$ mRNA levels and thyroid hormone binding in testis of different ages is shown in the *inset*. [Reproduced with permission from E. A. Jannini *et al.*: *Mol Endocrinol* 8:89–96, 1994 (118). © The Endocrine Society.]

(121). A similar Rev mRNA pattern has been observed in other rat tissues (108). During testis development $TR_{\alpha 1}$ is localized in the seminiferous epithelium but not in the interstitium, as shown by *in situ* hybridization (Fig. 3). Since $TR_{\beta 1}$ is absent at all ages, the testis is a unique naturally occurring mammalian model in which to study the effects of thyroid hormone mediated through $TR_{\alpha 1}$. In fact, this is the first *in vivo* confirmation of previous works with *in vitro* translated proteins (109), that $TR_{\alpha 1}$ binds its ligand and mediates thyroid hormone effects (see *Section II.C*). Furthermore, these data clarify the results that failed to detect thyroid hormone binding and biological effects when only mature testis was studied.

The localization of TR proteins in testis has produced somewhat discordant results. Using antibodies raised against the native rat hepatic receptor (122, 123) or $TR_{\alpha 1}$ and $TR_{\beta 1}$ aminoacidic sequences (124–128), positive immunostaining was seen in the testicular interstitium, in the nuclei of spermatogonia, spermatocytes, and mature spermatozoa from adult rat testis but, surprisingly, not in Sertoli cells. However, these findings contrast with the cited reports (106, 112–118) that adult testis contains neither $TR_{\alpha 1}$ nor $TR_{\beta 1}$ mRNAs and no detectable T_3 binding activity. Two different antisera against rat $TR_{\beta 1}$ failed to detect a positive signal in adult testis, in agreement with mRNA results (117, 129). Interestingly, using a $TR_{\alpha 2}$ antiserum, a positive reaction has been found in nuclei of spermatogonia during mitosis (117). As the spermatogonia mature into spermatids, they lose reactivity to $TR_{\alpha 2}$; thus, the presence of $TR_{\alpha 2}$ protein, which correlates with the presence of its mRNA (106, 113, 118), suggests an important role in differentiation of germ cells for this "ligand orphan" receptor isoform.

In summary, high affinity-low capacity TR sites were found in fetal, neonatal, and, at a lower level, in prepuberal, but not in adult, testis of the rat. The adult testis expresses exclusively the nonhormone binding isoforms α_2 and Rev, the former being localized in spermatogonia. Binding studies and *in situ* hybridization strongly indicate the Sertoli cell as

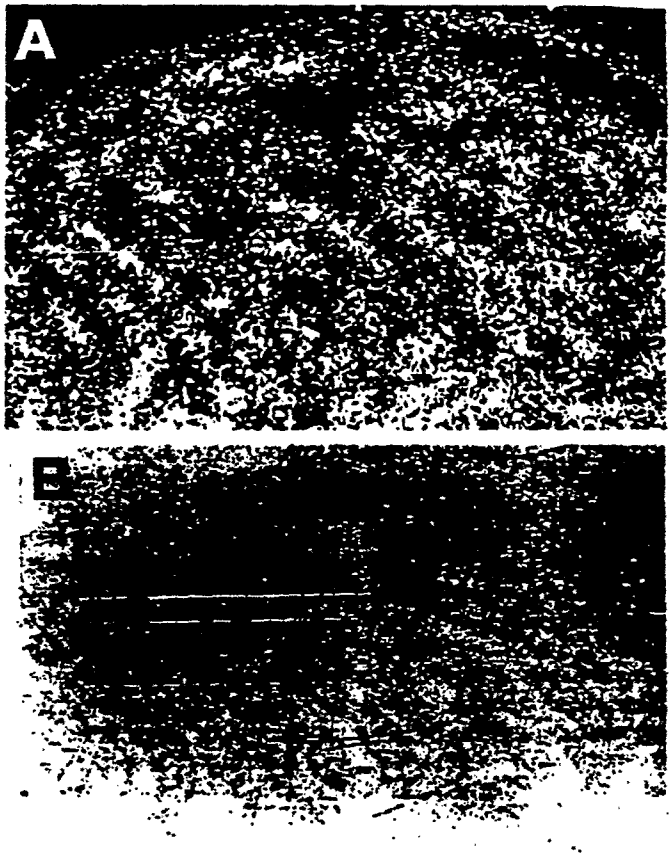

FIG. 3. Localization of TR_α mRNAs by *in situ* hybridization. The ^{35}S-labeled antisense riboprobe used in Fig. 2A ($TR_{\alpha 1}/TR_{\alpha 2}$) has been hybridized with testis sections from 15-day-old rats. The section is shown in dark (A) and light (B) field. Optical magnification, 40×. Note the localization of the positive signals in the seminiferous epithelium but not in the interstitium. For experimental procedures see Ref. 118.

the target of thyroid hormone action in the testis. More important, the characteristic ontogenetic profile of T_3 binding activity coincides with the unique expression of $TR_{\alpha 1}$ mRNA.

C. Biochemical effects of thyroid hormone in testis

Having established that the Sertoli cell, among testicular cell types, is the only cell that expresses functional TR, the effect of thyroid hormone on immature Sertoli cell functions has received major attention in recent years. In the seminiferous microenvironment, the mobilization of energy resources must be strictly controlled since the activity and survival of germ cells depend on the supply of factors produced by Sertoli cells (130). In this cell, FSH represents the main regulator of energy metabolism. It rapidly stimulates glucose transport and lactate production (131). In Sertoli cells from immature rats, thyroid hormone stimulates glucose transport by increasing the synthesis of glucose transporter GLUT1 mRNA, in a process requiring *de novo* protein synthesis (132). Furthermore, T_3 stimulates lactate production (133) while Sertoli cell cultures from immature animals made hypothyroid from birth with methimazole exhibit lower lactate production with respect to controls (134). Thus, in Sertoli cells, FSH and thyroid hormone might regulate glucose uptake at different levels, through both a fast membrane-signaling mechanism and a delayed action *via* the nuclear level, so that energy requirements of the developing germ cells can be met.

Other metabolic effects in the testis have been demonstrated upon thyroid hormone manipulation *in vivo*. In the early 1950s, Barker and Klitgaard (3) showed that T_4 did not affect oxygen consumption of adult testis. The classic assumption of the unresponsiveness of male gonad to thyroid hormone is derived from this observation. However, it has been recently demonstrated in Sertoli cells from prepuberal hypothyroid rats that the oxidative capacity and ATP content are lower, compared with coeval controls (135). T_4 treatment for 1 month increased the specific activity of isocitrate dehydrogenase (NADP+) in whole testis but lowered the activities of pyruvate kinase (78), ATP-citrate lyase, malate dehydrogenase, and malic enzyme (136), all testicular enzymes of the pyruvate/malate cycle involved in lipogenesis. Opposite results were obtained in thyroidectomized animals (137). However, part of these experiments (78, 136, 137) were done in animals older than 3 weeks, when there are no functional TRs available, or their level is very low.

Prepuberal Sertoli cells secrete insulin-like growth factor-I (IGF-I) (138), a mitogenic and differentiating factor, which may exert a paracrine (139) and/or autocrine (140) action in the regulation of Sertoli and germ cell functions. In prepuberal Sertoli cells, IGF-I release is stimulated *in vitro* by T_3 (141). The role of androgen binding protein (ABP), specifically produced by Sertoli cells, is to maintain a high local androgenic environment for developing germ cells (142). Its concentration increases in developing testis when TR declines in Sertoli cell (143). Furthermore, ABP is inhibited by T_3 treatment both at protein (144) and at mRNA levels (118). In adult testis, the relative steady state levels of ABP mRNA were similar in controls and in rats recovered from early induced transient hypothyroidism (145). Inhibin is a protein produced by the Sertoli cell with FSH-suppressing activity (146). In the prepuberal rat, its serum levels and mRNA accumulation are increased by *in vivo* (79) and *in vitro* T_3 treatment (120). In prepuberal transient hypothyroidism, immunoreactive inhibin levels are initially suppressed (87, 88), rise after cessation of goitrogen treatment, and are about 2-fold greater than normal during adulthood (82). In the same experimental conditions, inhibin-β_B mRNA shows a comparable profile (120). The correlation between inhibin response to thyroid manipulation and FSH serum levels in hyper- and hypothyroidism (see *Section III.A*) further suggests a gonadal site of action of thyroid hormone. Transferrin mRNA, a protein that delivers iron to spermatocytes and spermatids within the adluminal compartment of the seminiferous tubule (147), does not appear under thyroid hormone control either in prepuberal (118, 120) or adult rats (118, 120, 145). Androgen aromatization is a specific property of immature Sertoli cells and is catalyzed by an enzyme complex, termed aromatase, which is present in the endoplasmic reticulum. It represents an important marker of Sertoli cell differentiation and is strictly controlled by FSH *via* the stimulation of cAMP (148). Basal and FSH-induced aromatase activity in prepuberal rat Sertoli cells is inhibited *in vitro* (149) and *in vivo* (150) by T_3. Transient hypothyroidism also alters mRNA expression pattern of other important Sertoli cell proteins (120). The early expression of the Müllerian inhibiting substance mRNA, a member of the transforming growth factor-β gene family (151), is prolonged and the mRNA expression for clusterin, the most abundant Sertoli cell secretory product (152), is delayed. The altered developmental pattern of expression of specific genes in the seminiferous epithelium may reflect prolonged mitogenesis and delayed maturation of Sertoli cells in animals that are transiently hypothyroid (see *Section III.D*).

In summary, many functions of the Sertoli cell are under the control of thyroid hormone. *In vivo* or *in vitro* T_3 administration increases, at RNA and/or at protein level, glucose carrier units, IGF-I, and inhibin, while it decreases the aromatase activity and the production of ABP, without affecting transferrin. Hypothyroidism reduces oxidative capacity and increases inhibin levels. Finally, in euthyroid adult rats made hypothyroid during the neonatal period, an increase of Mullerian inhibiting substance and a decrease in clusterin has been observed. The biochemical effects of thyroid hormone manipulation demonstrate that the Sertoli cell is the main, if not the unique, direct target in the testis for thyroid hormone, and that the prepuberal period is the temporal frame for its action. The increased metabolic activity of the Sertoli cell caused by T_3 appears to be a prerequisite for the expansion of spermatogenesis. Thus, T_3 shares with FSH the role of pivotal regulator of the early phases of tubular development. The data discussed in the next paragraph further sustain this hypothesis.

D. Developmental effects of thyroid hormone in testis

The differentiation of Sertoli and germ cells *in utero* and their subsequent proliferation during fetal and perinatal life are complex events involving presently unknown signals for the initiation of differentiation from within the testis as well

as humoral factors from extratesticular sites. In the rat, the maximal proliferation of Sertoli cell occurs in late gestation and in the perinatal period (153), coinciding with the onset of fetal thyroid function (154), with the T_3 maximal binding capacity in the testis (111) and with the expression of FSH-induced ABP secretion, cAMP production, and aromatase activity (reviewed in Ref. 130). The number of dividing Sertoli cells decreases with increasing postnatal age and ceases before puberty (152); by this time the T_3-binding capacity (111) and $TR_{\alpha 1}$ mRNA expression in Sertoli cells (118) are significantly reduced, as is the responsiveness to FSH of the above mentioned biochemical markers of Sertoli cell maturation. The Sertoli cell number is the main determinant of final testicular size, and factors affecting this number will have effects on the volume and weight of the adult gonad. FSH is the main positive signal for Sertoli cell proliferation and is thought to be involved in the compensatory testicular hypertrophy after neonatal hemicastration or in immunization against estradiol, inhibin, or GnRH. Negative regulators of Sertoli cell division have been identified in substances produced by Leydig cells: testosterone and β-endorphin (reviewed in Refs. 130 and 155). The signal that stops Sertoli cell growth when adult size is reached, despite the continued presence of high levels of trophic hormones, remains unclear. Recent data on the action of T_3 on testis development indicate that thyroid hormone is dramatically involved in the regulation of ultimate testicular size by affecting the differentiation of the seminiferous epithelium.

It has been demonstrated both *in vivo* and *in vitro* that thyroid hormone directly regulates the early postnatal development of rat testis. *In vivo* administration of T_3 for 3 days during the first week of life causes a 60% increase in testis size (79, 80, 156). Furthermore, neonatal testis fragments cultured *in vitro* in the presence of thyroid hormone showed a significant increase in the size of seminiferous cords and in the number of gonocytes, concomitant with a decreased percentage of degenerating germ cells (Fig. 4) (80). A similar stimulatory effect on the seminiferous epithelium has been described also when thyroid hormone injection is started later but still in prepuberal life (75). In newborn rats, longer *in vivo* exposure to T_3 for 16 days further accelerates testis development by reducing the proliferative activity of Sertoli cells and gonocytes, anticipating lumen formation, and reducing the final weight of the adult testis (79, 157). On the contrary, in the adult testis, both *in vivo* and *in vitro* treatments with thyroid hormone did not induce any morphological modifications (80), thus confirming that the *critical window* of thyroid hormone effectiveness coincides with the prepuberal period. More than a direct effect on Sertoli cell proliferation rate, it appears that T_3 hastens the terminal differentiation of immature Sertoli cells into functional nonproliferating cells (79). In fact, *in vitro* T_3 treatment does not affect thymidine uptake and incorporation into DNA by Sertoli cells, even if thyroid hormone coincubation fully prevents the stimulatory effect of FSH on Sertoli cell mitogenesis (158). The increased number of gonocytes after T_3 treatment is related to an indirect effect of the hormone, because these cells do not express functioning TRs. Indeed, thyroid hormone stimulates Sertoli cells to secrete nutrients (*i.e.* lactate) essential for germ cell survival (159) and growth factors, such as IGF-I, which stimulate DNA synthesis in mitotic germ cells (160). Thus, the effects of T_3 on the germ line seem likely to result from a paracrine signal from the Sertoli cells (Fig. 5).

Although it was reported as early as 1923 (161), and confirmed in 1936 (162), that growth of testes and epididymides of young rats was retarded by thyroidectomy, a renewed interest has recently developed in controlling testicular size by thyroid hormone (155, 163, 164). Three different schedules of goitrogen treatment have been used. The antithyroid drug was given either chronically from birth to adulthood, or to the adult animal, or transiently for the first 3 weeks of life, allowing the recovery to euthyroidism before puberty. Each treatment affects testicular development differently. Chronic hypothyroidism produced by the administration of methimazole from birth to puberty induces a delay in the maturation of the seminiferous tubules and reduces their diameter and the number of germ cells per tubule, with increased degeneration and arrested maturation leading to a reduced final testis size (79, 84, 86, 156, 165, 166). In addition, Sertoli cells show retarded development, as indicated by a delay in the appearance of cytoplasmatic lipids and in the development of the tubular lumen (86). When 1-month-old prepuberal rats

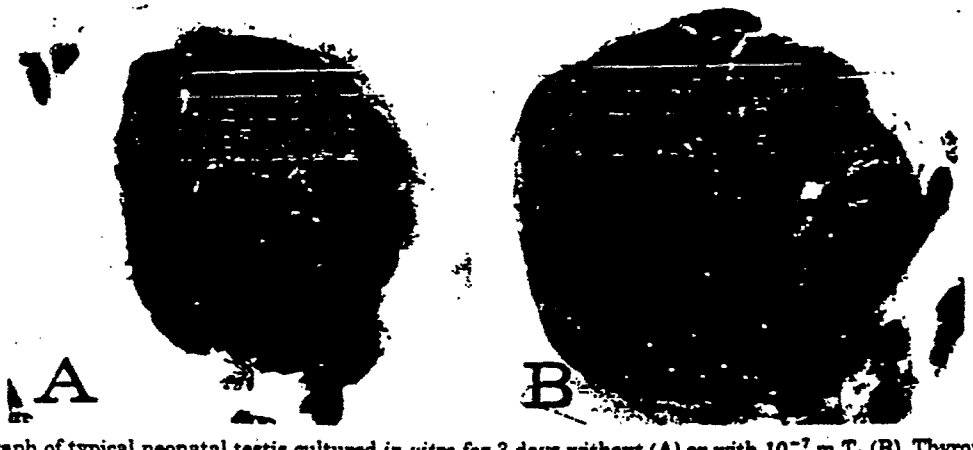

FIG. 4. Photomicrograph of typical neonatal testis cultured *in vitro* for 3 days without (A) or with 10^{-7} m T_3 (B). Thyroid hormone increases the diameter of seminiferous cords and the number of gonocytes (*filled arrow*) and decreases the rate of degenerating germ cells (*open arrow*). For experimental procedures see Ref. 80.

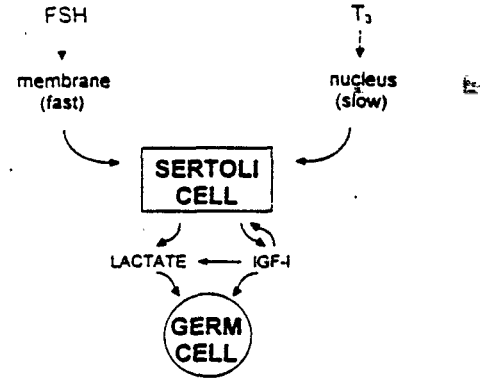

FIG. 5. Sertoli-germ cell interactions. The Sertoli cell is stimulated by FSH and T_3 to take up glucose and to produce lactate through a fast-membrane and a slow-nuclear signaling, respectively. The first does not require *de novo* protein and mRNA synthesis, while the second does. The lactate produced is essential for germ cell survival. Both hormones stimulate IGF-I synthesis by Sertoli cell, which can act as an autocrine factor to increase lactate production, and in a paracrine way to stimulate the replication of mitotic germ cells. Data were compiled from Refs. 130–134, 141, 159, and 160.

are thyroidectomized (167) or given goitrogens in adulthood (89, 90, 95, 96), testis growth and fertility are not affected, even if body weight is decreased by 50%. On the contrary, early induced transient hypothyroidism by the administration of the reversible goitrogen propylthiouracil (PTU), only for the first 3 weeks after birth, increases adult testis and reproductive organ size (87, 88, 93, 94, 163, 164, 166, 168–170) as well as sperm production efficiency (171). In particular, PTU treatments of various duration beginning at birth produce a graded increase in testis weight and sperm production. To be effective in the adult, the critical period of PTU treatment is the first postnatal week; treatments starting after this time are ineffective (94). Meisami *et al.* (166) confirmed this early postnatal period as critical for the PTU effect, although these authors found a slightly broader window of PTU effectiveness. Histologically, the area of the testis occupied by seminiferous tubules is equal in age-matched controls and in testes that underwent transient neonatal hypothyroidism. However, the mean seminiferous tubule diameter and length are increased in the PTU-treated animal. The number of leptotene spermatocytes and round spermatids is increased by 84% and 93%, respectively (145, 163, 164). This leads to an increased daily sperm production by 83% at 90 days of age, with maximal increase (140%) at 160 days after birth (171). Treated animals were also fertile and sired litters of normal size and without negative effects on postnatal development (93). Thus, transient hypothyroidism in the neonatal period appears to have no deleterious effects on reproductive functions. The same treatment retards the terminal differentiation of Sertoli cells (88) and increases their final number up to 157% (88, 145), delaying their differentiation from mitogenic to mature, nonproliferating state. Considering that the final number of germ cells is directly correlated to that of their *nurse* cell (172, 173), the increased Sertoli cell population may account for the augmented number of spermatogonia and, finally, of viable gametes. These effects account for the macroorchidism that occurs after prepuberal hypothyroidism. The manipulation of the thyroid hormone environment thus provides a means for producing increases in testis size and sperm production. The development of this system, if it could be extended to larger animals, might have a potentially significant economic impact.

The effects on rats made hypothyroid in the neonatal period and allowed to recover normal thyroid function by withdrawal of PTU at weaning are not due to the main regulators of seminiferous epithelium, since the serum levels of gonadotropins and testosterone remain unchanged or even depressed (86–88, 93, 94). Considering that TSH induces a mature morphology in cultured Sertoli cells (174) and affects Sertoli cell-differentiated functions (175), the possibility that these effects of the transient neonatal thyroid hormone deprivation are due to the increased TSH secretion cannot be ruled out. However, chronic hypothyroidism, which increases TSH from birth to adulthood, has opposite effects on seminiferous epithelium, decreasing final testis size and germ cell number (86), thus making this hypothesis unlikely. It has also been hypothesized that the resurgence of thyroid hormones in the period after PTU treatment may be responsible for the change that produces the observed testicular effects (168). The resumption of thyroid hormone secretion may affect a functionally *younger* and still responsive tissue that continues to complete maturation. This can be the case, considering that transient hypothyroidism delays the time-course of postnatal decline of nuclear T_3 receptor (80, 176) and TR_α mRNA (80, 120, 121).

In summary, thyroid hormone plays a pivotal role in the regulation of the terminal differentiation of Sertoli cells. T_3 administration in prepuberal life transiently increases testis size. Longer exposure to the hormone further accelerates testicular development by hastening the period of Sertoli cell proliferation, decreasing the number of Sertoli cell per testis, and hence causing a reduction in final testis size. Permanent hypothyroidism from birth to puberty also reduces the adult testis weight by retarding the maturation of Sertoli cells. This condition results in a delayed appearance of the seminiferous tubule lumen and in the inability of Sertoli cells to support advanced stages of germ cell development, with cellular degeneration and testicular atrophy. The reestablishment of euthyroidism before puberty allows Sertoli cells to fully mature and stimulates a higher rate of sperm production, leading to macroorchidism. The effects of various thyroid hormone manipulations on testis development are schematized in Fig. 6.

IV. Studies in Other Animals

Considerable work has been done to study the effects of various thyroid preparations and goitrogens on testis growth, semen characteristics, fertility, and seasonal reproduction of economically important animals, as well as of laboratory species. The effects of thyroid hormone on male reproduction in animals were reviewed in 1952 by Maqsood (177) and in 1970 by Gomes (178).

A. General studies

1. Monkeys. In rhesus monkeys, T_3 stimulates sperm adenylate cyclase and fructolysis (179). The impact of hyper- and

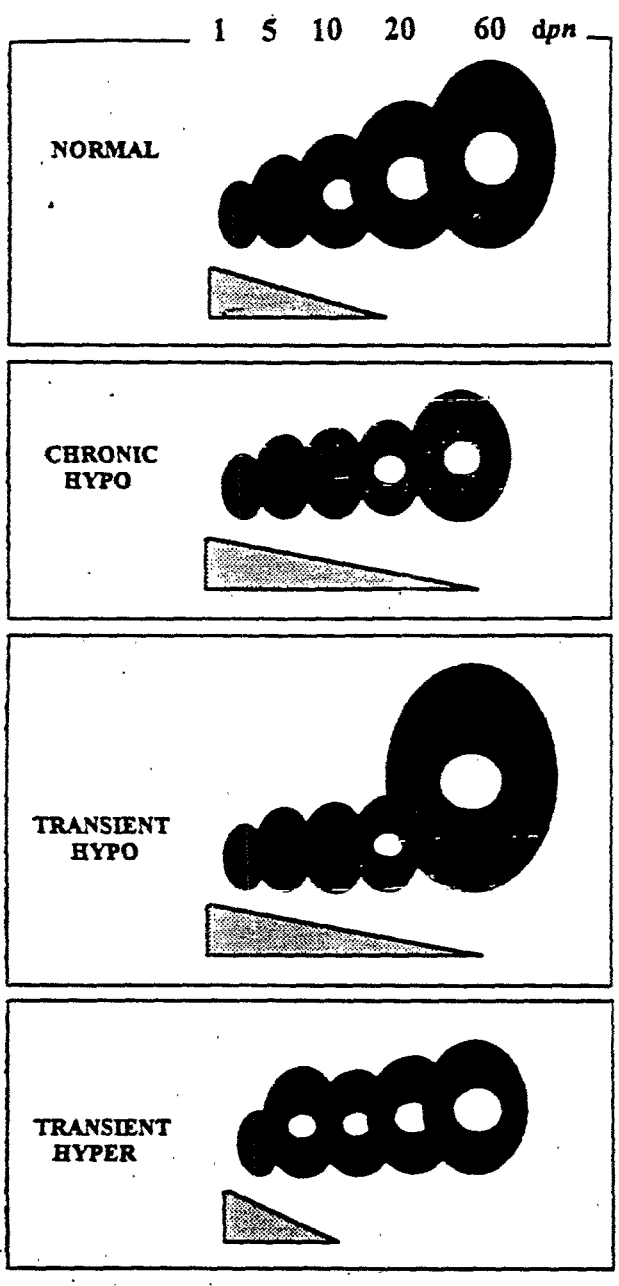

FIG. 6. Schematic representation of the effects of thyroid hormone manipulation on rat Sertoli cell proliferation, lumen formation, and testis size. *Chronic Hypo*, Methimazole given from birth to adulthood delays the cessation of Sertoli cell proliferative activity. The absence of the differentiative effect of T_3 delays the appearance of tubular lumen, leading to a reduced final testis size. *Transient Hypo*, PTU given from birth for 3 weeks, allowing the recovery to euthyroidism at weaning, delays the cessation of Sertoli cell proliferation. The resumption of thyroid hormone after PTU withdrawal allows Sertoli cell to fully mature and increases final testis size. *Transient Hyper*, Short term T_3 treatment increases testis size and hastens the appearance of tubular lumen. By prolonging T_3 administration, Sertoli cell proliferative activity ceases before control coeval animals, leading to a reduced final testis size. The area of the *triangles* indicates Sertoli cell proliferation; *light circles* indicate lumen formation and *dark ovals* indicate testis size from birth to adulthood. dpn, Days *post natum*. Data were compiled from Refs. 79, 80, 86, 88, 93, 94, 155, and 158.

hypothyroidism on testis in primates other than men is unknown. However, in male cynomologus monkeys made thyrotoxic by the administration of excess T_4, the MCRs of androgen and estrogen did not change significantly compared with control values, although the percentage of free testosterone fell and the concentration of SHBG rose significantly. The peripheral aromatization of androstenedione to estrone fell, but the aromatization of testosterone to estradiol was unchanged (180).

2. *Dogs.* Beagle puppies treated with PTU show a retardation in development of spermatogenesis that is compensated after puberty (181). If confirmed, these results resemble those previously discussed in the rat and can be explained in the same way.

3. *Pigs.* TRs in Sertoli cell have been characterized in prepuberal piglet (182). In these cells, T_4 *in vitro* dramatically enhances the production of transforming growth factor-β-like protein (183), which stimulates 2-deoxy-D-glucose uptake and lactate production in an autocrine way (184). A mechanism of action similar to that proposed above for the rat may also be involved in development of germinal epithelium in pigs.

4. *Cattle.* A slight increase in the conception rate has been reported in bulls fed thyroid extract (185), while T_4 at high doses was reported *in vitro* to inhibit respiration of spermatozoa and to stimulate glycolysis (186). In the male calf, thyroidectomy results in the complete absence of sexual drive in adulthood, which can be restored by thyroid therapy (187).

5. *Sheep and goats.* In lambs, the administration of T_4 before puberty results in a decrease in testis size and serum testosterone levels and in impairment of sexual development with alterations in LH pulse frequency (188). T_3-treated rams show improvement in semen quality, while their testes show active spermatogenesis and interstitial hypertrophy (189, 190). The administration of goitrogen for 1 yr to the young lamb prevents the onset of sexual maturity. Treated animals do not produce ejaculate. Histological examination of testes shows marked degenerative and atrophic changes (189). Furthermore, in thyroidectomized rams, a reduced sperm density and motility and an increased percentage of abnormal spermatozoa have been reported (191–193). Adult thyroidectomy in the same animal does not affect testis size or reproduction (188).

6. *Rodents.* In squirrels (194, 195) and rabbits (189, 190), mild hyperthyroidism has been reported to improve fertility. Severe prepuberal hypothyroidism in rabbits may result in delayed sexual maturity and decreased testis weight (189, 190). In mouse, transient hypothyroidism and T_3 treatment cause effects similar to those reported in the rat (see *Section III.D*). When administered to prepuberal mice in doses that are slightly greater than physiological, T_4 appears to shorten the time of testis development, and there is a tendency toward early maturation, while large doses of thyroid hormone result in a decrease in the final weight of the testes (196). Transient neonatal PTU treatment induces a delay in Sertoli cell arrest of proliferation and, after goitrogen withdrawal, a

30% increase in testis size and 50% increase in daily sperm production in the presence of normal testosterone serum levels (197). It has been suggested that this technique may be useful for increasing testicular size, sperm production, and fertility in various mutant mouse strains and transgenic mice in which these parameters are reduced. In male adult guinea pigs, no close relationship has been found between thyroid activity and reproduction. However, fertility is increased by T_4 administration and decreased by thyroidectomy (198). The same treatment does not affect the reproductive tract in adult male hamsters (199). However, it has been reported that neonatal PTU treatment at high doses produces an increase in adult testis size and sperm production (163, 164). The requirement for a higher dose of goitrogen may simply reflect a different metabolism of the hamster. However, this difference between rat/mouse and hamster emphasizes the individuality of each species, underscoring the potential difficulties when this model is extended to other species.

7. Avians. Feeding desiccated thyroid tissue or T_4 to chicken of various ages and breeds results in decreased final testis size, sperm activity, and fertility (200). It was reported that the same treatment stimulates testicular maturation in immature domestic ducks (201), while thyroidectomy prevents the normal growth of testis and penis (202).

8. Reptiles and amphibians. In the garden lizard, thyroidectomy causes gonadal regression, which can be overcome by thyroid hormone administration (203). It has also been demonstrated that thyroid treatment stimulates spermatogenesis in *Rana pipiens* (204) and in the toad *Bufo regularis* (205).

9. Fish. In chum salmon, T_3 concentrations in testis extracts were highest in the spermatogonium stage, decreased gradually during the spermatocyte and spermatozoon stage, and further decreased in the fully matured testis (206). This raises the possibility of a role of T_3 during early gamete and/or gonadal maturation in fish.

To summarize (Table 3), in the rat and in most of the animal species studied, thyroid hormone treatment early in life decreases the final testis size by stimulating testicular differentiation, while transient prepuberal hypothyroidism causes testicular overgrowth and sperm hyperproduction. If initiated later in life, both treatments have little or no effect on male reproduction. This confirms that the time *window* of thyroid hormone responsiveness is *critical* in a broader range of animals than rodents. In the animal species in which either T_3 nuclear binding or $TR_{\alpha 1}$ mRNA expression, or thyroid hormone direct actions have been demonstrated in testis, the described effects are to be ascribed to a nuclear and peripheral site of action.

B. Thyroid hormone effects on seasonal reproduction

The seasonal pattern of reproductive ability exhibited in animals across a considerable phylogenetic spectrum leads to alternating periods of reproductive activity and quiescence, correlated in the male with variations in testis size, hormonal

TABLE 3. Effects of thyroid hormone on testis function

Animal (age of treatment)	Effect (site of action)	Ref. No.
A. T_3 Treatment		
Rhesus monkey (a)	↑ Sperm adenylate cyclase, fructolisis	179
Pig (p)	↑ TGFβ (Sertoli cell)	183
Bull (a)	↑ Conception rate	185
Bull (a)	↓ Sperm respiration, ↑ glycolisis	186
Lamb (p)	↓ Final testis size	188
Ram (a)	↑ Sperm production	189
Squirrel (a)	↑ Conception rate	194, 195
Guinea pig (a)	↑ Conception rate	198
Hamster (a)	↔ Testicular function	199
Mouse (p)	↓ Final testis size	196
Chicken (p, a)	↓ Final testis size, sperm motility, fertility	200
Duck (p)	↑ Testicular maturation	201
Frog (p)	↑ Spermatogenesis	204
Toad (a)	↑ Spermatogenesis	205
B. Hypothyroidism		
Dog (p)	↓ Spermatogenesis	181
Calf (p)	↓ Sexual drive	187
Lamb (p)	↓ Puberty onset, testicular atrophy	189
Ram (p)	↓ Sperm motility and density	191–193
Ram (a)	↔ Testis size, fertility	188
Rabbit (p)	↓ Final testis size	189, 190
Guinea pig (a)	↓ Conception rate	198
Hamster (p, transient)	↑ Final testis size	163, 164
Mouse (p, a)	↓ Fertility	198
Mouse (p, transient)	↑ Final testis size, ↑ sperm production	197
Duck (p)	↓ Final testis size	202
Lizard (p, a)	↓ Testis size	203

p, Prepuberal; a, adult; ↓, decrease, ↑, increase, ↔, unchanged with respect to coeval controls. Hyper- and hypothyroidism have been chronically induced, except where otherwise indicated.

pattern, gamete production, mating behavior, and breeding capacity. Photoperiodism is the ability of the "endogenous clock" to respond to seasonal changes of the environment, perceived by the animals as changes in the light-dark ratio. Refractoriness is the involution of testis, spermatogenesis, and accessory organs due to some degree to a low light-dark ratio, as in the winter season (207). Such endogenicity is an important component of at least a portion of the annual reproductive cycle in many seasonal breeders.

The experiments in the rat and in other animal species summarized in the last paragraphs have been performed in fixed light cycles, *i.e.* abolishing the photoperiodism. Under these conditions, there is general agreement that adult male reproduction is insensitive to thyroid manipulation. In contrast, when seasons are mimicked experimentally and photorefractoriness taken into account, different results may be obtained.

1. *Deer and cattle.* In adult red deer stags, thyroid hormone is required for the expression of seasonal cessation of reproduction (208). At the time of the seasonal transition to the nonbreeding state, deer show an increase in circulating T_3 concentrations (209, 210). In these animals, thyroidectomy nearly abolished the nonbreeding season and hypothyroid stags retain full-grown testes throughout the year (210). Also in the ram, hypothyroidism overcomes the seasonal (photorefractory) inhibition of reproductive ability, abolishing the seasonal regression of testis (211).

2. *Mustelidae.* It has been reported that winter testicular regression is slower in thyroidectomized than in intact mink (212).

3. *Avians.* In 1940, Woitkewitsch reported a role for the thyroid gland in the seasonal reproduction of avians (213). Comparable effects have been recently demonstrated. When starlings are thyroidectomized before the breeding season, testes remain large indefinitely (214–216). Similar results have been obtained in finch (217) and quail (218). Conversely, thyroid hormone replacement in hypothyroid animals reinstates photorefractoriness (219). Furthermore, T_3 treatment mimics naturally occurring photorefractoriness, leading to a rapid testicular collapse (220). As fowls become refractory, there is an increase in central production of GnRH (221). Thyroid hormone treatment (222) causes a significant increase in gonadotropin secretion (223, 224) as well.

In summary, the temporal frame of thyroid hormone effectiveness in regulating seasonality is much broader than when nonseasonal reproduction is considered. Instead of testicular atrophy, as in nonseasonal conditions, chronic thyroid hormone deficiency inhibits the photoperiodic regression of testis, and the gonads appear increased in size with respect to control animals. In these conditions, the inhibition of seasonal regression of testis is *bona fide* and is believed to be due to the disruption of seasonal rhythms through the central, neuroendocrine pathway. Since photoperiodism is under central neuroendocrine control (207), it is conceivable that thyroid hormone may act, with other factors, at this level in regulating seasonality (225). The influence of thyroid status on seasonal breeding provides a rich source of research opportunities, many of which can only be glimpsed at this time. Furthermore, taking into account the regulatory role of T_3 in seasonality, it is necessary to consider photoperiodism when the effects of thyroid hormone manipulation on testicular development are studied.

V. Conclusion

The classic assumption that adult male gonad is unresponsive to thyroid hormone is no longer tenable. The seminiferous epithelium of prepuberal testis can now be considered a novel thyroid hormone-responsive tissue. This is confirmed by the presence in Sertoli cells from various animal species of T_3 nuclear binding that correlates with $TR_{\alpha 1}$ mRNA expression, and by the specific effects elicited by T_3 *in vitro* and *in vivo* on several tubular functions.

T_3 directly promotes the differentiation of the prepuberal Sertoli cell, with concomitant changes in its proliferation and secretory activity. Thus, thyroid hormone must be considered together with FSH as a major endocrine regulator of seminiferous epithelium development. In addition, the data concerning seasonal reproduction indicate that the activity of thyroid gland is required for normal expression of circannual cycles of reproductive activity across a wide phylogenetic spectrum (216). The responsiveness of testis to thyroid hormone has two remarkable characteristics. First, the testis is responsive to thyroid hormone only during a limited period of time that coincides with perinatal and prepuberal age. Second, the testis, at least in the rat, is the tissue that exclusively expresses the α_1-isoform of TR. This allows the use of testis as a naturally occurring model for studying the role of $TR_{\alpha 1}$ *in vivo* in the modulation of gene expression and cellular functions.

Even if the application of animal data to human pathology is questioned, valuable information can be obtained when animal data are properly interpreted. It is, in fact, of extreme interest to observe that in the inherited syndrome of generalized resistance to thyroid hormones, characterized by the exclusive mutation of TR_β form, abnormalities of gonadal function have been observed only in one of 146 males recently reviewed by Refetoff *et al.* (226). This strongly suggests that, also in human testis, $TR_{\alpha 1}$ is the unique TR expressed and TR_β is not involved in the altered reproductive functions associated with hypo- and hyperthyroidism.

The lack of relevant abnormalities of male gonadal function in hyper- and hypothyroidism that occurs in adulthood may be related to the unresponsiveness of the testis to thyroid hormone at this time. However, when hypothyroidism affects boys in the early postnatal period, macroorchidism and absence of androgenic testicular secretion may occur. These findings indicate that, similar to brain maturation, a normal thyroid hormone *milieu* in the perinatal period is required for normal testicular development. Finally, physicians should carefully investigate gonadal abnormalities of children at major risk of hypothyroidism, such as those in iodine-deficient environments, or those receiving antithyroid therapies.

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